

Biomonitoring of heterocyclic aromatic amine metabolites in human urine

W.G. Stillwell^a, R.J. Turesky^b, R. Sinha^c, P.L. Skipper^a, S.R. Tannenbaum^{a, d,*}

^a*Division of Bioengineering and Environmental Health, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

^b*Nestlé Research Center, Nestec Ltd., 1000 Lausanne 26, Switzerland*

^c*Division of Cancer Epidemiology and Genetics, NCI, Rockville, MD 20892, USA*

^d*Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

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Abstract

Human exposure to heterocyclic aromatic amines such as MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline) may be monitored by measuring the levels of the heterocyclic aromatic amine in urine. In order to investigate the contribution of *N*-oxidation to the metabolism of MeIQx in vivo, we developed a biomonitoring procedure for the analysis and quantification of the *N*²-glucuronide conjugate of 2-hydroxyamino-3,8-dimethylimidazo[4,5-*f*]quinoxaline in human urine. Subjects (*n* = 66) in the dietary study ingested a uniform diet of cooked meat containing known amounts of MeIQx, and urine was collected after consumption of the test meal. A method based on solid-phase extraction and immunoaffinity separation was used to isolate *N*²-(β-1-glucosiduronyl)-2-hydroxyamino-3,8-dimethylimidazo[4,5-*f*]quinoxaline and its stable isotope-labeled internal standard from urine. The isolated conjugate was converted to the deaminated product 2-hydroxy-3,8-dimethylimidazo[4,5-*f*]quinoxaline by treatment with acetic acid under moderate heating. 2-Hydroxy-3,8-dimethylimidazo[4,5-*f*]quinoxaline and the [²H₃]methyl analog were derivatized to form the corresponding 3,5-*bis*(trifluoromethyl)benzyl ether derivatives and quantified by capillary gas chromatography–negative ion chemical ionization mass spectrometry employing selected ion monitoring procedures. The amounts of *N*²-(β-1-glucosiduronyl)-2-hydroxyamino-3,8-dimethylimidazo[4,5-*f*]quinoxaline recovered in urine collected 0–12 h after the test meal accounted for 2.2–17.1% of the ingested dose, with a median value of 9.5%. The variability in the proportion of the dose excreted among the subjects may be reflective of several factors, including interindividual variation in the enzymic activity of CYP1A2 and/or conjugation reactions of the *N*-hydroxylamine metabolite with *N*-glucuronosyltransferase(s). © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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* Corresponding author. Massachusetts Institute of Technology, Cambridge, MA 02139, USA. Tel.: 617-253-3729; fax: 617-252-1787.

E-mail address: srt@mit.edu (S.R. Tannenbaum)

Abbreviations: HAA, heterocyclic aromatic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; [²H₃]MeIQx, 2-amino-3-[²H₃]methyl-8-methylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; *N*-OH-MeIQx, 2-hydroxyamino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 2-nitro-MeIQx, 2-nitro-3,8-dimethylimidazo[4,5-*f*]quinoxaline; *N*-OH-MeIQx-*N*²-glucuronide, *N*²-(β-1-glucosiduronyl)-2-hydroxyamino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 2-OH-MeIQx, 2-hydroxy-3,8-dimethylimidazo[4,5-*f*]quinoxaline; CYP1A2, cytochrome P4501A2; NCI-GC/MS, negative ion chemical ionization-gas chromatography/mass spectrometry; SIM, selective ion monitoring

1. Introduction

Meats cooked at high temperature give rise to a number of heterocyclic aromatic amines (HAAs) which can be metabolically activated to mutagenic/carcinogenic intermediates. MeIQx and PhIP represent two of the major HAAs found in cooked meats, and considerable effort has been focused on the determination of these compounds and their metabolites in human studies. Controlled dietary studies have provided quantitative data on the relationship between the levels of MeIQx and PhIP in urine and the ingested dose [1–5]. The results demonstrated that the HAAs are excreted in a dose-related manner, thus establishing a basis for studies in determining the comparable dietary exposure in humans ingesting unrestricted diets. Recently, assessments of HAA exposure have been conducted in a limited number of examinations [6–8]. Significant interethnic differences in the urinary excretion levels of MeIQx have been observed [6], indicating that daily exposure to HAAs in the diet varies considerably and is dependent on several factors, including dietary preferences and methods of cooking.

Dietary studies have found that the urinary levels of unchanged MeIQx [9] or unchanged plus amine-conjugated MeIQx [4] are inversely related to CYP1A2 activity, thus showing that CYP1A2 function is an important factor in the disposition of MeIQx. A recent investigation characterizing MeIQx metabolism in humans confirmed the presence of *N*-OH-MeIQx-*N*²-glucuronide in urine [10]. This metabolite, derived via CYP1A2 oxidation, accounted for 1.4–10% of the dose in these individuals. In order to assess the *in vivo* capacity of humans to *N*-oxidize MeIQx, we developed a biomonitoring procedure for the analysis of the *N*²-glucuronide conjugate of 2-hydroxyamino-MeIQx in urine. In addition, information on the excretion levels of this metabolite would establish a basis for the extent of *N*²-glucuronidation reactions in the disposition of *N*-OH-MeIQx.

2. Materials and methods

2.1. Study design

A detailed account of the study design, partici-

pating subjects, and dietary protocol has been described elsewhere [9,11].

2.2. Chemicals and reference metabolites

MeIQx, [²H₃]MeIQx, and [2-¹⁴C]-MeIQx were purchased from Toronto Research Chemicals (Ontario, Canada). The synthetic procedures have been described in detail [12]. Briefly, 2-hydroxyamino-3,8-dimethylimidazo[4,5-*f*]quinoxaline was prepared by the reduction of 2-nitro-MeIQx. *N*²-(β-1-glucosiduronyl)-2-hydroxyamino-3,8-dimethylimidazo[4,5-*f*]quinoxaline and its [²H₃]methyl and [2-¹⁴C] analogs were biosynthetically prepared from the corresponding 2-hydroxyamino-MeIQx derivatives using rat liver S-9 homogenates [13]. 2-OH-MeIQx was synthesized by treating *N*-OH-MeIQx-*N*²-glucuronide with an organic solution of aqueous acetic acid at 60°C for 6 h or by base treatment of 2-nitro-MeIQx. The products were purified by high-pressure liquid chromatography and characterized by UV/visible and mass spectrometry. Monoclonal antibodies raised against MeIQx were used in the preparation of immunoaffinity columns [14].

2.3. Isolation and analysis of *N*-OH-MeIQx-*N*²-glucuronide

Urine samples (8 ml) were spiked with the stable isotope-labeled internal standard [²H₃]*N*-OH-MeIQx-*N*²-glucuronide. Bond-Elut Certify II solid-phase extraction, immunoaffinity purification, and acidic hydrolysis of *N*-OH-MeIQx-*N*²-glucuronide to form 2-OH-MeIQx were used in the analytical procedure. The 2-OH-MeIQx-containing fraction was derivatized with 3,5-bis(trifluoromethyl)benzyl bromide for analysis by NCI-GC/MS. The column used was a DB-35ms fused-silica capillary. Quantification was carried out by selected ion monitoring for the molecular anions at *m/z* 440 for 2-OH-MeIQx and *m/z* 443 for its [²H₃]methyl analog.

The recovery of 2-OH-MeIQx derived from *N*-OH-MeIQx-*N*²-glucuronide was determined by spiking urine samples with a known amount of ¹⁴C-labeled *N*-OH-MeIQx-*N*²-glucuronide. The samples were carried through the entire procedure described above and the amount of radiolabeled 2-OH-MeIQx in the derivatized extracts was assessed by scintillation counting.

3. Results and discussion

The MeIQx content in the high-temperature cooked meat was 9.0 ng/g, and the quantity of meat eaten by each of the subjects was 3.1–4.4 g meat/kg of body weight. The total amount of meat eaten by each person ranged from 180–328 g, with a median value of 248 g.

3.1. Synthesis of 2-hydroxy-MeIQx

When *N*-OH-MeIQx-*N*²-glucuronide and 2-nitro-MeIQx were hydrolyzed with acetic acid and base, respectively, a single major product was observed. Mass spectral and UV/visible analyses of the product are consistent with a structure in which the 2-amino group of MeIQx has been replaced by a hydroxyl group.

3.2. Analysis of urine extracts

The isolation scheme was developed to provide a specific and sensitive method for biomonitoring analysis of *N*-OH-MeIQx-*N*²-glucuronide in urine. Solid-phase extraction combined with immunoaffinity column chromatography allowed a high degree of separation of the conjugate from other components in urine. Hydrolysis of the conjugate to form 2-OH-MeIQx followed by derivatization and quantification by NCI-GC/MS was found to be a rapid and efficient method for the analysis of multiple samples. Good detection limits in the low picogram range were noted for the 3,5-*bis*(trifluoromethyl)benzyl derivative of 2-OH-MeIQx in the SIM mode. The lower detection limit of the analysis was 80 pg of *N*-OH-MeIQx-*N*²-glucuronide per 8 ml of urine. Blank urine samples analyzed under the same conditions showed no detectable presence of *N*-OH-MeIQx-*N*²-glucuronide. The overall recovery of 2-OH-MeIQx derived from *N*-OH-MeIQx-*N*²-glucuronide in urine was determined to be, on the average, $55 \pm 9\%$ (\pm SD) based on analysis of the ¹⁴C-labeled compounds.

The amounts of *N*-OH-MeIQx-*N*²-glucuronide found in the 0–12 h urine, for all subjects, ranged from 78 to 915 ng, with an average value of 391 ± 138 ng (\pm SD). Linear regression analysis showed a highly significant correlation ($r = 0.49$; $P < 0.0001$) between the amounts of *N*-OH-MeIQx-

*N*²-glucuronide excreted in urine versus the ingested dose of MeIQx. The excretion levels of *N*-OH-MeIQx-*N*²-glucuronide in the 0–12 h urine ranged from 2.2–17.1% of the ingested dose, with a median value of 9.5%.

In summary, the immunoaffinity separation procedure is highly selective for the purification of *N*-OH-MeIQx-*N*²-glucuronide and enables detection of the metabolite at levels of 80 pg/8 ml of urine. Our results show that *N*-OH-MeIQx-*N*²-glucuronide is readily detectable in human urine after consumption of MeIQx and that the amounts excreted correlate with the ingested dose of MeIQx. The urinary levels of *N*-OH-MeIQx-*N*²-glucuronide ranged from 2.2 to 17.1% of the dose, indicating the variability and extent of *N*-oxidation and *N*²-glucuronidation reactions in the metabolism and disposition of MeIQx in humans.

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